Filopodia: **Fickle fingers of cell fate?**

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Epithelial cells often produce extensions, known variously as filopodia, cell feet or cytonemes, which can extend across many cell diameters to directly contact non-adjacent cells. Do they function in morphogenesis, cell–cell signaling or both?

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Current Biology 1999, 9:R655–R657
http://biomednet.com/elecref/09609822009R0655

0960-9822/99/$ – see front matter

Once in a while, a simple new discovery sends old biologists furtively scurrying around in their filing cabinets, looking for those dusty old files full of unpublished micrographs, muttering "How could I have missed that?" "It must be in those pictures somewhere!" "If only…" The recent observation by Ramirez-Weber and Kornberg [1] of huge numbers of long thin filopodia on *Drosophila* imaginal disc cells is one of these discoveries. These cell processes — which the authors call 'cytonemes' — measure only 0.2 µm across, so they are near the limit of resolution for light microscopy, but they can be up to 800 µm long and appear to make direct contact with cells far away in the epithelium. Similar cell processes have been seen in other insects and in sea urchin embryos (Figure 1), but the fact that thin filopodia are now showing up in a favorite model system for genetic studies of pattern formation is stimulating widespread rethinking about how cells communicate with each other.

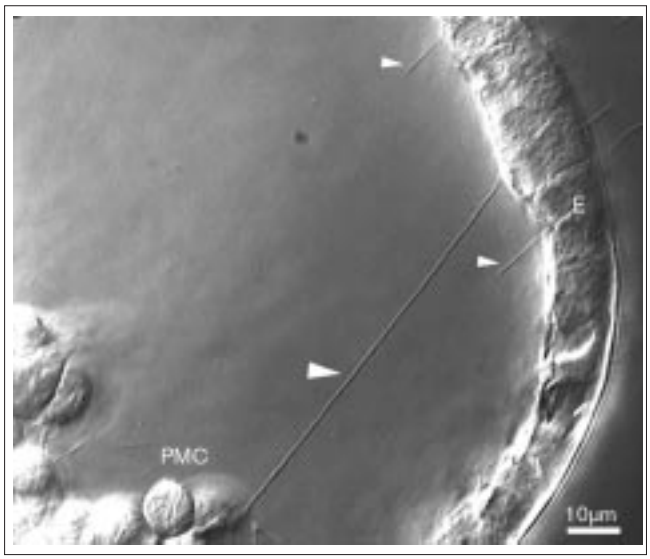
The actin-rich but tubulin-free cytonemes were discovered by fluorescence microscopy of imaginal discs, the 'prepatterned' larval structures that evert to form the adult fly body parts. Certain regions of the discs — gene expression domains or mitotic recombination clones — were producing the marker green fluorescent protein (GFP), which made cell projections from the GFP-positive areas visible against the dark background. The projections were not randomly distributed or oriented, but emanated from cells at the anterior and posterior regions of the disc, and extended across many cell diameters to end in the region of the anterior–posterior compartment boundary. Unfortunately, these will o' the wisps disappeared upon fixation, so they could not be examined in sections or by electron microscopy.

Thin filopodia similar to cytonemes have been described in a variety of developing systems, and they have usually been thought to have mechanical roles. Locke [2] found them in various cell types in two insects — the skipper butterfly *Calpodes* and the blood-sucking bug *Rhodnius*. These microfilament-containing structures — 70–100 nm wide and 10–30 µm long — were not present on undisturbed cells, but formed almost instantly on the basal surface of cells from which the basal lamina had been removed, or that had been separated from their neighbors. Locke's interpretation was that their function was to sample the environment and to provide the tension necessary to bring cells back in contact with each other or with the basal lamina. Basal filopodia seem to function in a similar way in the formation of the insect tracheal system. When cells are deprived of oxygen they extend long thin filopodia that contact air-filled tracheoles in nearby areas and pull them toward the oxygen-deprived cell, thereby correcting the oxygen deprivation [3]. Some of these filopodia are no more than 50 nm wide but over 100 µm long; unlike cytonemes, they contain microtubules as well as microfilaments.

In addition to thin filopodia, insect epithelial cells often produce more substantial processes from their basal ends, called cell feet, that might also function in direct interactions between non-adjacent epithelial cells. Locke and Huie [4] developed a method for staining a random subset of cells in an epithelial sheet, which made the feet easy to distinguish against a background of unstained cells. They showed that the feet extended for several cell diameters, that they were preferentially oriented, and that their extension was controlled by the molting hormone 20-hydroxyecdysone. Nardi and Magee-Adams [5] then used this technique to stain the scale-forming cells of the pupal moth wing. They found that these cells are initially arranged irregularly, but that at a certain stage in pupal development they align into straight rows by moving laterally within the cell sheet. At exactly the time when these movements are occurring, the scale-forming cells extend elaborate basal cell feet across many cell diameters to contact other scale-forming cells in the same row and even in other rows. These contacts could be responsible for directing the cell movements that result in precise alignment of scales on the adult wing.

Ramirez-Weber and Kornberg [1] have discovered some of the signals that induce the formation of cytonemes on imaginal disc cells. From experiments with fragments of discs brought together *in vitro* it seems that cytonemes are induced only by tissue fragments containing the anterior–posterior boundary, and that the attraction is dependent on the product of the *hedgehog* gene (a signalling molecule known to have a number of important functions

Figure 1



A primary mesenchyme cell (PMC) in a sea urchin gastrula extends a long thin filopodium (large arrowhead) across the blastocoel to contact a surface ectoderm cell (E). The ectodermal cells also extend shorter filopodia (small arrowhead). (Image courtesy of Scott Fraser.)

in *Drosophila* development). Furthermore, the effect of anterior–posterior boundary cells could be replaced by fibroblast growth factor (FGF), even though FGF is produced throughout the wing disc and not just at the anterior–posterior boundary region [1]. Coincidentally, the *Drosophila* FGF homolog Branchless is also involved in another example of cell extension: it is required for the formation of long, thin processes by the terminal tracheal epithelial cells, which form the fine (< 1 μm wide) tubular tracheoles that deliver air to the internal tissues [6]. Branchless, acting through its receptor Breathless, is also responsible for inducing development of larger-scale branches of the tracheal system [7].

It is quite surprising that cytonemes have not previously been seen on the epithelial cells in ultrastructural studies of imaginal discs; this is presumably a result of their propensity to disappear upon fixation. Cellular processes have, in fact, been described on imaginal disc cells *in vitro*, where they are thought to be involved in cellular reaggregation [8]; but these processes contain tubulin and are probably related to cell feet rather than thin filopodia. Filopodia have also been described on the mesenchymal adepithelial cells, the muscle precursors in imaginal discs, especially those that had not yet been joined into columns [9]. This suggests they might have a function in bringing cells together during early stages of muscle morphogenesis.

Ramirez-Weber and Kornberg [1] go beyond the previous speculations about the roles cell processes might play in morphogenesis and cell spacing. They suggest that cytonemes may allow long-range signaling between cells in the imaginal disc epithelium, thereby contributing to the elaboration of the spatial pattern. This adds new complexity to the continuing debate over whether pattern formation in imaginal discs involves 'long-range signaling'. The most likely long-range signaling event in imaginal discs, and the one most likely to involve cytonemes, is the one mediated by Decapentaplegic (Dpp), a member of the transforming growth factor-β (TGF-β) family of cell signaling molecules (see [10] for review).

In response to prior signaling events mediated by the Hedgehog protein, a narrow stripe of Dpp expression forms along the anterior side of the anterior–posterior compartment boundary in the imaginal wing disc, and this expression is required for normal patterning and growth, as well as for cell survival, in the disc. Some elegant genetic experiments have shown that Dpp can control gene expression and pattern formation at a great distance from its source. First, ectopic production of Dpp in a somatic clone reorganizes the wing pattern over large areas outside the clone [11]. Second, expression of Dpp target genes — *spalt* and *optomotor blind* — was found to be induced in cells far from the area where Dpp was produced, either in its normal location or ectopically in somatic clones [12]. Different target genes were induced over different ranges from the apparent source, suggesting that they respond to different Dpp concentrations in a gradient formed by diffusion of Dpp away from its source.

An alternative to the diffusion gradient model is a sequential relay mechanism, in which Dpp interacts with a receptor on neighboring cells, which are thereby induced to produce either Dpp or a different morphogen, which in turn activates their neighbors, and so on. Genetic evidence argues strongly against such a model, however. Local expression of an activated Dpp receptor in a somatic clone was found to activate downstream genes only within the region of expression, and did not have the kind of non-autonomous effects outside the clone that were seen with Dpp ectopic expression [13]. The diffusion gradient model has thus received strong support.

There is, however, no direct evidence that the Dpp protein can diffuse within the tissue to form a concentration gradient, and it is difficult to imagine how diffusion could establish a reproducible gradient within a columnar epithelium. Furthermore, as other TGF-β family members have been shown to bind to extracellular matrix [14], thus restricting their movement from the source of production, Dpp is also unlikely to diffuse far. The discovery of cytonemes provides a possible way around these difficulties, because cells at a distance from the Dpp source could contact the source directly via their cytonemes. Some of the phenotypes produced by ectopic

Dpp expression [11], however, show that the protein can cause a long-range alteration of pattern, even if it is not being produced at an anterior–posterior boundary. It will now be necessary to determine whether cytonemes are induced in these experimental situations.

Long, thin, straight filopodia — 0.2–0.4 μm diameter and > 80 μm long — containing actin have also been documented by high-resolution Nomarski imaging of gastrulating sea urchin embryos, where they also appear to mediate direct, long-range cell interactions that control patterning [15]. In these embryos, the ectodermal cells of the blastula wall have been shown to influence both the number and size of spicules produced by the primary mesenchyme cells [16]. Studies using time-lapse video microscopy have shown extensive production of both thick and thin filopodia at this time. The highly dynamic, thin filopodia are produced by the primary mesenchyme cells and contact ectodermal cells across the blastocoel cavity (Figure 1); they are also extended by secondary mesenchyme cells and ectodermal cells. Like Kornberg's group, Miller *et al.* [15] have suggested that some signaling events previously thought to be mediated by diffusible signals may in fact be mediated by direct contact between signaling and responding cells via thin filopodia.

Most studies of filopodia and cell feet have suggested a role in morphogenesis, or more specifically in guiding the movements of cells toward or away from each other. Filopodia seem to explore surfaces of other cells, identify appropriate sites for adhesion, and then guide the cell body in subsequent morphogenetic events. This is the case for most of the studies of cell feet and filopodia discussed earlier, as well as many other examples from migratory non-epithelial cells. The cytonemes in imaginal discs are not, however, in appropriate positions or orientations for such a role [1].

Ramirez-Weber and Kornberg's [1] suggestion that imaginal disc cytonemes have a signaling role, presumably mediated by Dpp, is a fascinating idea, but of course it raises many new questions. First of all, are the cytonemes transmitting positional information? In the wing disc, cells would have to be assigned different fates depending on their anterior–posterior positions, so cytonemes would have to receive different signals and so presumably contact different cells in the signal-generating Dpp source. But this would imply that cells already had different properties, so why would they need to extend cytonemes? It will be important to determine whether these structures extend in a directed manner, which would imply that positional information was already established in the disc, or whether, like other filopodia, they explore at random until they encounter an appropriate target site. Secondly, what kind of signal might be transmitted? It is difficult to imagine that subtle quantitative differences of signal could be transmitted over long thin filopodia, but qualitatively different signals might be possible. And lastly, how are cytonemes induced? Ramirez-Weber and Kornberg [1] found that they are induced by fragments of the leg or antenna discs that contain an anterior–posterior border, but that they could not be induced by fragments of eye discs, which do not have such a border. While this limits the potential significance of cytonemes to anterior–posterior-related patterning, it also adds substantially to the already numerous mysteries about why anterior–posterior compartments exist and how they function in pattern formation.

The text-book picture of epithelial cells as polygonal bricks is doubtless a gross oversimplification. These cells can produce amazing processes, probably with equally amazing dynamics. They can reach out and touch someone — but what they are saying to each other is still a well-kept secret.

Acknowledgements

The author's work is supported by grants from NIH, NSF, and the Human Frontiers Science Program.

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